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RESEARCH ARTICLE

Coumaroyl Quinic Acid Derivatives and Flavonoids from Immature Pear (*Pyrus pyrifolia* Nakai) Fruit

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Abstract Fourteen compounds were isolated from 60% ethanol extracts of immature pear (*Pyrus pyrifolia* Nakai cv. Chuhwangbae) fruit using Amberlite XAD-2 column HPLC with guided DPPH radical scavenging assay. Based on MS and NMR analysis, the isolated compounds were identified as 5-*O*-*trans*-caffeoyl quinic acid methyl ester (**1**), malaxinic acid (**2**), 5-*O*-*trans*-*p*-coumaroyl quinic acid methyl ester (**3**), 5-*O*-*cis*-*p*-coumaroyl quinic acid methyl ester (**4**), 5-*O*-*trans*-*p*-coumaroyl quinic acid (**5**), *trans*-*p*-coumaric acid (**6**), methyl *cis*-*p*-coumarate (**7**), methyl *trans*-*p*-coumarate (**8**), 3,5-*O*-dicaffeoyl quinic acid (**9**), (-)-epicatechin (**10**), (*S*)-(+)-2-*cis*-abscisic acid (**11**), isorhamnetin 3-*O*-β-D-galacto-pyranoside (**12**), isorhamnetin-3-*O*-β-D-glucopyranoside (**13**), and isorhamnetin 3-*O*-α-L-rhamnopyranosyl (1→6)-*O*-β-D-glucopyranoside (**14**). Six compounds (**1**, **2**, **6**, **9**, **10**, and **13**) were identified previously, but other compounds (**3-5**, **7**, **8**, **11**, **12**, and **14**) were isolated for the first time from pear.

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Introduction

Pear (*Pyrus* spp.) belongs to the Rosaceae family and Pomaceae tribe and has been cultivated in 81 countries with temperate climates (1), and more than 60 species are widely distributed around the world (2,3). Fresh pear fruit is commonly used for table purposes and in processed foods such as juices, purees, jellies, and jams (4). In Asian countries such as Korea, pear has traditionally been used as an herbal medicine to treat constipation, diuresis, cough, and flu (5). Investigations on pear chemical constituents such as sugars, organic acids, amino acids, and fatty acids have been performed (6). In addition, the main chemical constituents in pear fruit have been identified as arbutin, chlorogenic acid, catechins, and procyanidins (5,7-9). However, previous studies focused on investigating the constituents of European pears rather than those in Asian pears (7,8,10,11). Studies of secondary metabolites in pears have been promoted through the development of instrumental analyses such as HPLC-photo diode array, GC-MS, and LC-MS. Many constituents contained in European pears have been identified in previous research (12). However, the constituents contained in Asian pears have not been identified sufficiently (12), although a large variety of compounds contained in Asian pears is comparable to those contained in European pears (12). It might be ascribable to the absence of standard of constituents contained in Asian pears because the structure of compounds contained in Asian pears has not been clarified. Additionally, existing data suggest that the constituents contained in Asian pears

and their composition may be largely different from those in European pears. Understanding of the chemical constituents of Asian pear fruits may provide variety information on plant physiology and biological activity of pear cultivars. Studies on a few chemical constituents in Asian pears have been reported (13). Also isolated 7 phenylpropanoid malate derivatives and 8 phenolic compounds from *P. pyrifolia* Nakai cv. Chuhwangbae pear fruit, which is one of the most highly consumed pear fruits in Korea (14,15). However, it was considered that more systematic studies on the chemical constituents contained in Asian pear fruits are additionally required. It is generally known that the constituent content is higher in immature fruits than in mature fruits (16,17). Therefore, use of immature fruits may be more useful than using mature fruits to identify constituents. Furthermore, low quality immature pear fruits are generally handpicked during the early stage after florescence to harvest pears of high quality and the handpicked immature pear fruits are discarded (18). Therefore, this study tried to isolate and identify the compounds contained in immature pear fruits in this study.

Materials and Methods

Materials and chemicals Pear (*Pyrus pyrifolia* Nakai cv. Chuhwangbae) was cultivated in Naju and its immature fruit was collected after the 35 days of florescence in May 2011. The immature fruit was immediately frozen in dry ice, moved to the laboratory, and stored at −70°C until use. The samples were certified by Prof. W.-S. Kim (Laboratory of Pomology, College of Agriculture and Life Science, Chonnam National University, Korea). Methanol (MeOH) and acetonitrile (MeCN) were purchased from Fisher Scientific Korea Ltd. (Seoul, Korea). DPPH and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol-d_4 (CD_3OD) was obtained from Acros Organics (Fair Lawn, NJ, USA). EtOH and ethyl acetate (EtOAc), used for extraction, and HCl were purchased from Duksan Pure Chemical Co., Ltd. (Ansan, Korea).

DPPH radical scavenging assay on TLC Free radical scavenging activity of the fractions obtained from Amberlite XAD-2 column chromatography and HPLC was determined by spraying a stable radical, DPPH, on a TLC (Silica gel 60 F_{254}, 0.25-mm thickness; Merck, Darmstadt, Germany), according to the modified method of Takao *et al.* (19). Briefly, each fraction was spotted on the plate and developed with EtOAc/BuOH=6:4 (v/v). After spraying a 200 μM DPPH EtOH solution, fractions that were visualized as a decolorization of the spot were regarded as antioxidative active.

Isolation of antioxidants from immature pear fruit Immature pear fruit (8 kg, fresh weight) was homogenized in 60% EtOH (68 L) using a homogenizer (BM-2 Nissei bio-mixer; Nihonseiki Kaiseiki Ltd., Tokyo, Japan). After a 24 h extraction at room temperature, the homogenate was filtered under vacuum through no. 2 filter paper (Whatman International, Maidstone, UK). The residue was extracted 2 additional times using the same procedure. The filtrates were combined and concentrated *in vacuo* at 38°C with a rotary evaporator (N-2N; Eyela, Tokyo, Japan). The 60% EtOH extracts (560 g) were suspended in H_2O (3 L, pH 3.0 by 1 N HCl) and partitioned with EtOAc (3 L, 5 times) to give the EtOAc-acidic soluble fraction. The EtOAc-acidic soluble fraction (38.7 g) was fractionated by column (90× 900 mm) chromatography on Amberlite XAD-2 gel (20-60 mesh; Supelco, Bellefonte, PA, USA), and elution was performed with H_2O/EtOH solutions (10:0, 8:2, 4:6, 0:10, v/v, each 6 L). Fraction F (H_2O/EtOH=4:6, v/v) was subjected to HPLC (SPD-M20D; Shimadzu, Kyoto, Japan) equipped with a Shim-pack Prep-ODS (H) kit (5 μm, 20×250 mm; Shimadzu). The elution was performed with a linear gradient of 40% MeOH (pH 2.65 by TFA, eluent A) and 60% MeOH (eluent B) starting with 100% A, increasing to 100% B for 40 min, and then holding at 100% B for 60 min. Flow rate was 9.9 mL/min and detection was carried out at a wavelength of 254 nm. Five fractions (F3-F7) were further purified by HPLC with a RP-Amide column C16 (10×250 mm, Supelco). The elution was performed with a linear gradient of 15% MeCN (pH 2.65 by TFA, eluent A) to 30% MeCN (eluent B) starting with 100% A, increasing to 100% B for 30 min, and then holding at 100% B for 40 min. Flow rate was 4.5 mL/min, and the eluate was monitored at 254 nm.

Structural analysis NMR spectra were obtained with INOVA 500 and 600 spectrometers (Varian, Walnut Creek, CA, USA) using tetramethylsilane as an internal standard in CD_3OD. All mass spectra were acquired on a hybrid ion-trap time-of-flight mass spectrometer (Shimadzu) equipped with an electrospray ionization (ESI) source (ESI-MS). Accurate masses were corrected by calibration using sodium trifluoroacetate clusters as internal references. Sample solutions were prepared by dissolving each sample in a solution of MeOH to a final concentration of 50 μg/μL.
Compound **1**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.52 (1H, d, *J*=16.0 Hz, H-7'), 7.04 (1H, d, *J*=1.8 Hz, H-2'), 6.95 (1H, dd, *J*=8.3, 1.8 Hz, H-6'), 6.78 (1H, d, *J*=8.3 Hz, H-5'), 6.22 (1H, d, *J*=16.0 Hz, H-8'), 5.27 (1H, m, H-5), 4.13 (1H, m, H-3), 3.73 (3H, s, -OCH_3), 3.73 (1H, dd, *J*=7.0, 4.5 Hz, H-4), 2.18 (2H, m, H-6), 2.00 (2H, m, H-2).
Compound **2**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.85 (1H, dd, *J*=8.8, 2.0 Hz, H-6), 7.79 (1H, d,

J=2.0 Hz, H-2), 7.18 (1H, d, *J*=8.8 Hz, H-5), 5.35 (1H, m, H-2'), 5.03 (1H, d, *J*=7.5 Hz, H-1"), 3.90 (1H, dd, *J*=12.0, 2.0 Hz, H-6"b), 3.71 (1H, dd, *J*=12.5, 2.0 Hz, H-6"a), 3.55-3.39 (6H, m, H-1', 2"-5"), 1.75 (3H, s, H-4'), 1.73 (3H, s, H-5').

Compound **3**: Pale yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.67 (1H, d, *J*=16.0 Hz, H-7'), 7.48 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.81 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.38 (1H, d, *J*=16.0 Hz, H-8'), 5.26 (1H, m, H-5), 4.07 (1H, m, H-3), 3.71 (3H, s, -OCH_3), 3.68 (1H, dd, *J*=8.0, 3.0 Hz, H-4), 2.19-1.98 (4H, m, H-2, 6); ESI-MS (negative) *m/z* 351.0 $[M-H]^-$.

Compound **4**: Pale yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.65 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.86 (1H, d, *J*=13.0 Hz, H-7'), 6.75 (2H, d, *J*=8.5 Hz, H-3', 5'), 5.73 (1H, d, *J*=13.0 Hz, H-8'), 5.16 (1H, m, H-5), 4.14 (1H, m, H-3), 3.74 (1H, dd, *J*=8.0, 3.0 Hz, H-4), 3.69 (3H, s, -OCH_3), 2.19-1.98 (4H, m, H-2, 6); ESI-MS (negative) *m/z* 351.0 $[M-H]^-$.

Compound **5**: Pale yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.69 (1H, d, *J*=16.0 Hz, H-7'), 7.45 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.81 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.29 (1H, d, *J*=16.0 Hz, H-8'), 5.29 (1H, m, H-5), 4.15 (1H, m, H-3), 3.74 (1H, dd, *J*=8.0, 2.5 Hz, H-4), 2.22- 2.00 (4H, m, H-2, 6); ESI-MS (positive) *m/z* 339.0 $[M+H]^+$.

Compound **6**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.67 (1H, d, *J*=15.8 Hz, H-7), 7.48 (2H, d, *J*=8.5 Hz, H-2, 6), 6.81 (2H, d, *J*=8.5 Hz, H-3, 5), 6.37 (1H, d, *J*=15.8 Hz, H-8); ESI-MS (negative) *m/z* 163.0 $[M-H]^-$.

Compound **7**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.65 (2H, d, *J*=8.5 Hz, H-2, 6), 6.81 (1H, d, *J*=13.0 Hz, H-7), 6.75 (2H, d, *J*=8.5 Hz, H-3, 5), 5.89 (1H, d, *J*=13.0 Hz, H-8), 3.72 (3H, s, -OCH_3); ESI-MS (negative) *m/z* 176.9 $[M-H]^-$.

Compound **8**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.66 (1H, d, *J*=15.8 Hz, H-7), 7.48 (2H, d, *J*=8.5 Hz, H-2, 6), 6.81 (2H, d, *J*=8.5 Hz, H-3, 5), 6.38 (1H, d, *J*=15.8 Hz, H-8), 3.77 (3H, s, -OCH_3); ESI-MS (negative) *m/z* 176.9 $[M-H]^-$.

Compound **9**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.62 (1H, d, *J*=16.0 Hz, H-7'), 7.58 (1H, d, *J*=16.0 Hz, H-7"), 7.06 (2H, br. s, H-2', 2"), 6.97 (2H, d, br. d, *J*=8.3 Hz, H-6', 6"), 6.79 (1H, d, *J*=8.3 Hz, H-5'), 6.78 (1H, d, *J*=8.3 Hz, H-5"), 6.35 (1H, d, *J*=16.0 Hz, H-8"), 6.26 (1H, d, *J*=16.0 Hz, H-8'), 5.79 (1H, d, *J*=8.5 Hz, H-5'), 5.78 (1H, d, *J*=8.5 Hz, H-5"), 5.43 (1H, m, H-3), 5.38 (1H, br. s, H-5), 3.98 (1H, dd, *J*=7.0, 3.5 Hz, H-4), 2.34-2.15 (4H, m, H-2, 6).

Compound **10**: ^{1}H-NMR (CD_3OD, 500 MHz) δ 6.93 (1H, d, *J*=1.0 Hz, H-2'), 6.79 (1H, br. d, *J*=9.0 Hz, H-6'), 6.73 (1H, d, *J*=9.0 Hz, H-5'), 5.79 (2H, br. s, H-6), 5.76 (2H, br. s, H-8), 4.70 (1H, br. s, H-2), 4.45 (1H, m, H-3), 3.16-2.91 (2H, m).

Compound **11**: ^{1}H-NMR (CD_3OD, 600 MHz) δ 7.78 (1H, d, *J*=16.2 Hz, H-4), 6.25 (1H, d, *J*=16.2 Hz, H-5), 5.92 (1H, s, H-3'), 5.75 (1H, s, H-2), 2.53 (1H, d, *J*=16.8 Hz, H-5'b), 2.18 (1H, d, *J*=16.8 Hz, H-5'a), 2.04 (3H, d, *J*=1.2 Hz, H-6), 1.92 (3H, d, *J*=1.2 Hz, H-7'), 1.06 (3H, s, H-8'), 1.02 (3H, s, H-9'); ^{13}C-NMR (CD_3OD, 150 MHz) δ 201.2 (C-4'), 166.9 (C-1), 166.7 (C-2'), 151.3 (C-3), 139.1 (C-5), 129.5 (C-4), 127.7 (C-3'), 119.6 (C-2), 80.7 (C-1'), 50.8 (C-5'), 43.0 (C-6'), 24.8 (C-9'), 23.7 (C-8'), 21.4 (C-6), 19.7 (C-7'); ESI-MS (positive) *m/z* 287.1 $[M+Na]^+$.

Compound **12**: Dark yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.87 (1H, d, *J*=1.5 Hz, H-2'), 7.61 (1H, dd, *J*=8.8, 1.5 Hz, H-6'), 6.90 (1H, dd, *J*=8.8, 1.5 Hz, H-5'), 6.43 (1H, br. s, H-8), 6.22 (1H, br. s, H-6), 5.27 (1H, d, *J*=7.5 Hz, H-1"), 4.18 (1H, dd, *J*=12.0, 6.0 Hz, H-6"b), 3.94 (3H, s, -OCH_3), 3.72 (1H, dd, *J*=12.0, 2.5 Hz, H-6"a), 3.49-3.39 (4H, m, H-2"-5"); ESI-MS (negative) *m/z* 477.0 $[M-H]^-$.

Compound **13**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.88 (1H, d, *J*=1.8 Hz, H-2'), 7.61 (1H, dd, *J*=8.5, 1.8 Hz, H-6'), 6.91 (1H, d, *J*=8.5 Hz, H-5'), 6.43 (1H, d, *J*=2.0 Hz, H-8), 6.22 (1H, d, *J*=2.0 Hz, H-6), 5.22 (1H, d, *J*=7.5 Hz, H-1"), 3.95 (3H, s, -OCH_3), 3.38-3.49 (5H, m, H-2"-6").

Compound **14**: Yellow powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.94 (1H, br. s, H-2'), 7.62 (1H, br. d, *J*=8.0 Hz, H-6'), 6.91 (1H, d, *J*=8.0 Hz, H-5'), 6.39 (1H, br. s, H-8), 6.20 (1H, br. s, H-6), 5.24 (1H, d, *J*=7.0 Hz, H-1"), 4.53 (1H, br. s, H-1'"), 3.98-3.24 (9H, m, H-2"-6", 2'"-5'"), 3.95 (3H, s, -OCH_3), 1.10 (3H, d, *J*=6.0 Hz, H-6'"); ESI-MS (positive) *m/z* 647.2 $[M+Na]^+$.

Results and Discussion

Purification and isolation of antioxidants from immature pear fruit The 60% EtOH extracts (560 g) of immature pear fruit (8 kg f.w.) were suspended in H_2O (pH 3.0) and partitioned with EtOAc to give the EtOAc-soluble acidic layer (38.7 g). The EtOAc-soluble acidic layer (38.7 g) was fractionated by Amberlite XAD-2 column chromatography with stepwise elution of the H_2O/EtOH mixtures. An aliquot of each fraction was developed on TLC and then sprayed with a DPPH radical EtOH solution (200 mM). Most of the fractions (A-H) obtained showed DPPH radical scavenging activity. In particular, fraction F [H_2O/EtOH=4:6 (v/v), 5.96 g] showed the highest DPPH radical scavenging activity among the 8 fractions. Therefore, fraction F (1.5 g) was further purified by ODS-HPLC to give 7 fractions [F1 (t_R 11.1 min, 36.5 mg, **1**), F2 (t_R 12.3 min, 1.0 mg, **2**), F3 (t_R 14.8 min, 20.2 mg), F4 (t_R 18.1 min, 8.2 mg), F5 (t_R 19.3 min, 9.6 mg), F6 (t_R 22.1 min, 10.2 mg), F7 (t_R 23.7 min, 17.2 mg)]. Of them, 5 fractions (F3-7) were

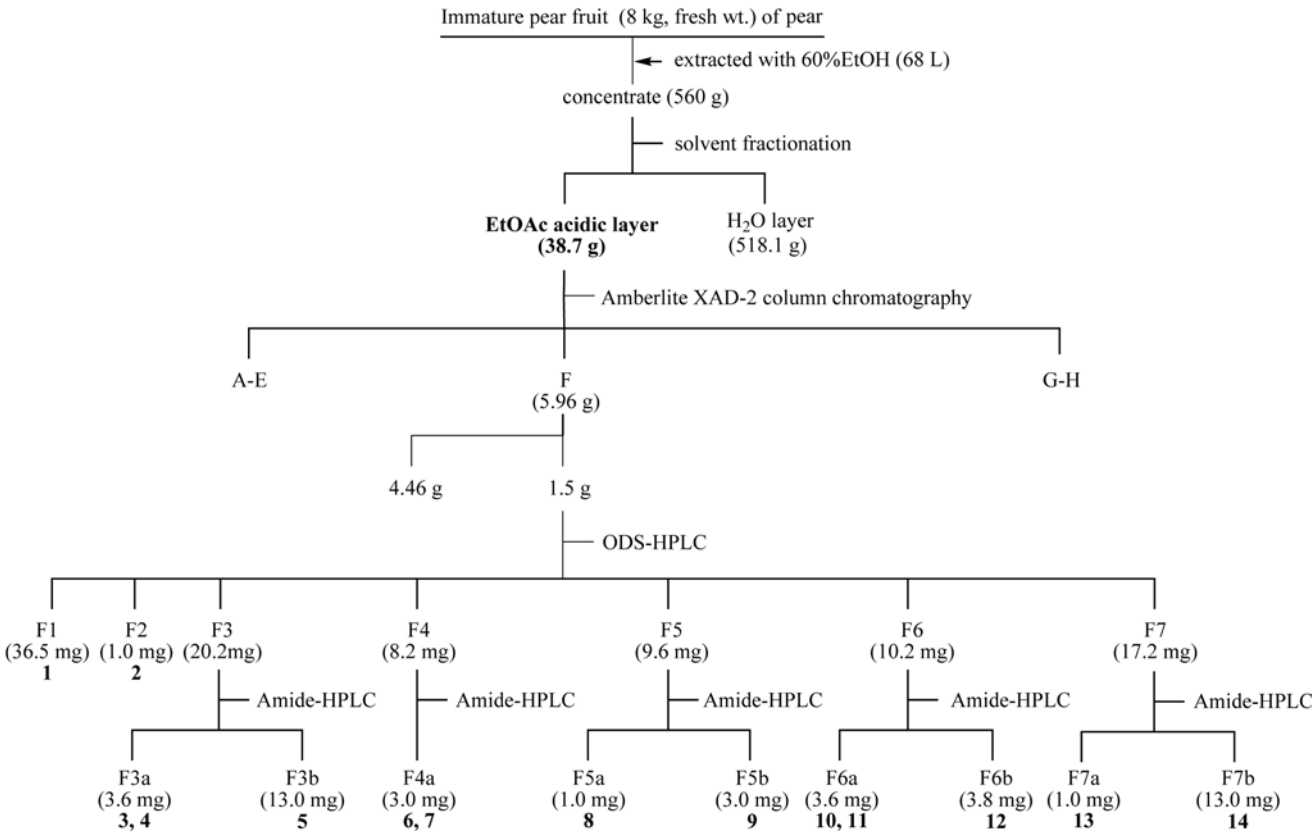

Fig. 1. Isolation and purification procedure for the isolated compounds from the ethyl acetate-acidic layer of the 60% ethanol extract from immature pear fruit.

further purified on an RP-Amide column. That is, F3a (a mixture of **3** and **4**, t_R 16.4 min, pale yellow powder, 3.6 mg) and **5** (t_R 14.6 min, pale yellow powder, 13.0 mg) from fraction F3, F4a (a mixture of **6** and **7**, t_R 17.6 min, yellow powder, 3.0 mg) from fraction F4, **8** (t_R 23.4 min, yellow powder, 1.0 mg) and **9** (t_R 27.1 min, yellow powder, 3.0 mg) from fraction F5, F6a (a mixture of **10** and **11**, t_R 28.9 min, 3.6 mg) and **12** (t_R 30.4 min, dark yellow powder, 3.8 mg) from fraction F6, and **13** (t_R 32.1 min, yellow powder, 1.0 mg) and **14** (t_R 35.2 min, green powder, 13.0 mg) from fraction F7 were purified, respectively. The isolation and purification procedure of the 14 compounds is illustrated in Fig. 1. The structure of the purified compounds was elucidated by NMR and MS analyses.

Identification of the isolated compounds Of the 14 isolated compounds, 5 were identified as 5-*O*-*trans*-caffeoyl quinic acid methyl ester (**1**), malaxinic acid (**2**), 3,5-dicaffeoyl quinic acid (**9**), (-)-epicatechin (**10**), and isorhamnetin 3-*O*-β-D-glucopyranoside (**13**), based on a comparison of their NMR and MS data reported in a previous study (15).

The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of F3a showed evidence of a mixture of 2 coumaroyl quinic acid methyl ester derivatives. The proton signals of 2 compounds displayed an intensity ratio of approximately 55:45 (main compound, **3**; minor compound, **4**). The signals assignable to methyl quinate in the 2 compounds were detected with a very similar pattern and a difference in chemical shift of <0.1 ppm. In addition, the ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **3** showed the presence of *para*-substituted benzene ring proton signals [δ 7.48 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.81 (2H, d, *J*=8.5 Hz, H-3', 5')] and olefinic double bond proton signals [δ 7.67 (1H, d, *J*=16.0 Hz, H-7'), 6.38 (1H, d, *J*=16.0 Hz, H-8')]. In particular, the coupling constant value (*J*=16.0 Hz) of the olefinic double bond proton signals indicated that the double bond was *trans*. Therefore, the partial structure of **3** was suggested to be *trans p*-coumaric acid. Additionally, 2 methylene proton signals [δ 2.19-1.98 (4H, m, H-2, 6)], 3 oxygenated methine proton signals [δ 4.07 (1H, m, H-3), 3.68 (1H, dd, *J*=8.0, 3.0 Hz, H-4), 5.26 (1H, m, H-5)], and a methoxyl proton signal at δ 3.71 (3H, s, -OCH_3)] confirmed quinic acid. Therefore, compound **3** was suggested to be 5-*O*-*trans*-*p*-coumaroyl quinic acid methyl ester. The ^{1}H-NMR spectrum of **3** was consistent with that of the 5-*O*-*trans*-*p*-coumaroyl quinic acid methyl ester isolated from *Cistus laurifolius* (20) (Fig. 2). The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **4** was very closely related to that of **3** except for the olefinic double bond proton signals [δ 6.86 (1H, d, *J*=13.0

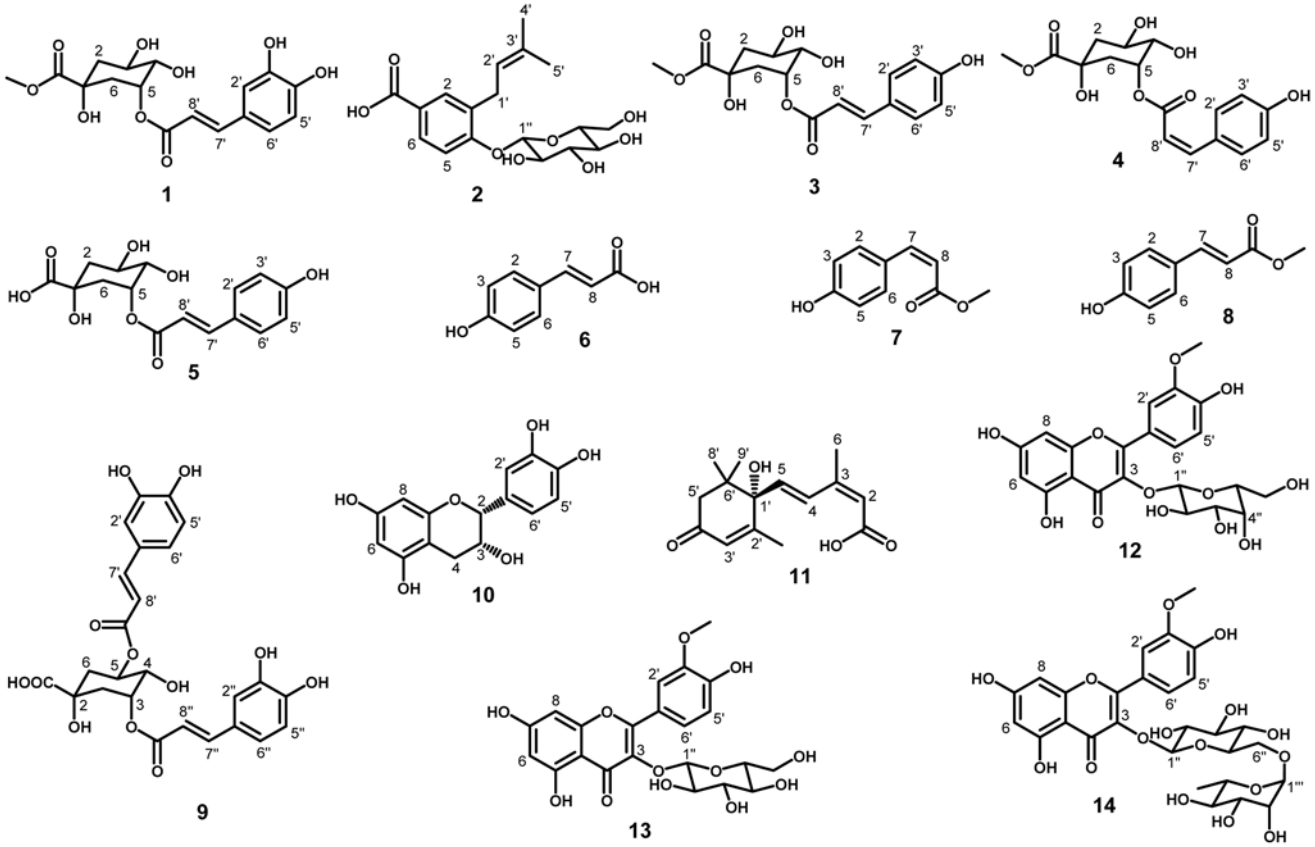

Fig. 2. Structures of isolated compounds from ethyl acetate-acidic layer of the immature pear fruit 60% ethanol extract.

Hz, H-7'), 5.73 (1H, d, *J*=13.0 Hz, H-8')]. That is, the *J* values of the olefinic double bond assignable to **4** ensured that the olefinic double was in the *cis* configuration. Therefore, compound **4** was suggested to be *cis-p*-coumaroyl quinic acid methyl ester. However, the conjugated position between quinic acid and coumaroyl groups was unclear. Therefore, the NMR data of **4** was compared with those of known coumaroyl quinic acid methyl ester derivatives. The ^{1}H-NMR spectrum of **4** agreed with that of the 5-*O*-*cis*-*p*-coumaroyl quinic acid methyl ester isolated from *Onobrychis viciifolia* (21). In addition, the molecular weight of this compound was determined to be 352 by the pseudomolecular ion peak *m/z* 351.0 $[M-H]^-$ in the ESI-MS (negative) spectrum. Therefore, **3** and **4** were unambiguously identified as 5-*O*-*trans*-*p*-coumaroyl quinic acid methyl ester and 5-*O*-*cis*-*p*-coumaroyl quinic acid methyl ester (Fig. 2), respectively.

The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **5** was closely related to that of **3** except for the absence of a methoxyl group proton signal. Therefore, compound **5** was suggested to be 5-*O*-*trans*-*p*-coumaroyl quinic acid. Additionally, the molecular weight (338) of **5** was confirmed by a signal corresponding to the compound from the ESI-MS (positive) data (pseudomolecular ion peak at *m/z* 339.0 $[M+H]^+$). Therefore, compound **5** was identified as 5-*O*-*trans*-*p*-coumaroyl quinic acid by comparing the ^{1}H-NMR spectrum as reported previously (22) (Fig. 2).

The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of F4a also showed evidence of a mixture of 2 compounds, which were observed with an unequal proton signal intensity at a ratio of 9:1 (main compound, **6**; minor compound, **7**). The ^{1}H-NMR spectrum of **6** was closely related to that of *trans*-*p*-coumaric acid as the partial structure of **3**. The presence of *para*-substituted aromatic ring proton signals [δ 7.48 (2H, d, *J*=8.5 Hz, H-2, 6), 6.81 (2H, d, *J*=8.5 Hz, H-3, 5)] and *trans*-form double bond proton signals [δ 7.67 (1H, d, *J*=15.8 Hz, H-7), 6.37 (1H, d, *J*=15.8 Hz, H-8)] revealed that **6** was a *trans*-*p*-coumaric acid. In addition, the ^{1}H-NMR spectrum of **6** agreed with that of *trans*-*p*-coumaric acid isolated from *gochujang* (23). The molecular weight of **6** was 164 due to the pseudomolecular ion *m/z* 163.0 $[M-H]^-$ in the ESI-MS (negative) spectrum. Therefore, compound **6** was unambiguously identified as *trans*-*p*-coumaric acid (Fig. 2).

The ^{1}H-NMR spectrum of **7** showed a very similar pattern to that of **6**. However, the *J* value of the olefinic double bond proton signals assignable to **7** at δ 6.81 (H-7) and 5.89 (H-8) was 13.0 Hz, which was distinct from that (*J*=15.8 Hz) of **6,** and a methoxyl proton signal was additionally observed at δ 3.72 (3H, s, -OCH_3). The

molecular weight of this compound was 178 due to the pseudomolecular ion *m/z* 176.9 $[M-H]^-$ in the ESI-MS (negative) spectrum. Therefore, compound **7** was unambiguously identified as methyl *cis-p*-coumarate with agreement of the ^{1}H-NMR spectrum as reported previously (23) (Fig. 2).

The ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **8** was very closely related to that of **6**, including the chemical shifts and *J* values of the *para*-substituted benzene ring and olefinic double bond proton signals of the *trans* form. However, the ^{1}H-NMR spectrum of **8** showed an additional methoxyl proton signal [δ 3.77 (3H, s, $-OCH_3$)]. The molecular weight of this compound was 178 due to a pseudomolecular ion peak at *m/z* 176.9 $[M-H]^-$ in the ESI-MS (negative) spectrum, which indicated that **8** possessed one more methyl group than that of **6**. The ^{1}H-NMR data were in agreement with those reported previously (24). Therefore, compound **8** was identified as methyl *trans-p*-coumarate (Fig. 2).

The molecular weight of **11** was 264 based on the pseudomolecular ion of *m/z* 287.1 $[M+Na]^+$ observed on its ESIMS (positive) spectrum. The ^{13}C-NMR (CD_3OD, 125 MHz) spectrum of **11** revealed the presence of 15 carbon signals, including 2 carbonyl carbons [δ 201.2 (C-4'), 166.9 (C-1)], 6 sp^2 carbons [δ 166.7-119.6], and 7 sp^3 carbons [δ 80.7-19.7], suggesting a sesquiterpene. These ^{13}C-NMR data were supported by the ^{1}H-NMR (CD_3OD, 600 MHz) spectrum of **11**. That is, the presence of 4 methyl proton signals [δ 2.04 (3H, d, *J*=1.2 Hz, H-6), 1.92 (3H, d, *J*=1.2 Hz, H-7'), 1.06 (3H, s, H-8'), and 1.02 (3H, s, H-9')], a methylene proton signal [δ 2.53 (1H, d, *J*=16.8 Hz, H-5'b) and 2.18 (1H, d, *J*=16.8 Hz, H-5'a)], 4 olefinic double bond proton signals [δ 7.78 (1H, d, *J*=16.2 Hz, H-4), 6.25 (1H, d, *J*=16.2 Hz, H-5), 5.92 (1H, s, H-3'), and 5.75 (1H, s, H-2)] was observed in the ^{1}H-NMR spectrum. Therefore, **11** was suggested to be abscisic acid based on the MS and 1D-NMR spectra. Additionally, this structure was confirmed by heteronuclear single-quantum correlation and heteronuclear multiple-bond correlation spectroscopy. The ^{1}H- and ^{13}C-NMR spectra of **11** agreed with those of (*S*)-(+)-2-*cis*-abscisic acid, as reported previously (25). Therefore, compound **11** was identified as (*S*)-(+)-2-*cis*-abscisic acid (Fig. 2).

The ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **12** was very closely related to that of **13**, which has already been identified from mature pear. Compound **13** contained glucose as a sugar moiety, whereas **12** contained galactose. Therefore, the ^{1}H-NMR spectrum of **12** was compared with that of isorhamnetin glycosides and it was consistent with the isorhamnetin 3-*O*-β-D-galactopyranoside isolated from blue woodruff (*Asperula arvensis*), as reported previously (26) (Fig. 2). The molecular weight of this compound was 478 based on the pseudomolecular ion *m/z* 477.0 $[M-H]^-$ in the ESI-MS (negative) spectrum. Therefore, compound **12** was identified as isorhamnetin 3-*O*-β-D-galactopyranoside.

The ^{1}H-NMR (CD_3OD, 500 MHz) spectrum of **14** was closely related to that of **12** except for the presence of proton signals assignable to 1 rhamnose moiety, including 2 anomeric protons [δ 5.24 (1H, d, *J*=7.0 Hz, H-1"), 4.53 (1H, br. s, H-1"')], 1 methyl proton [δ 1.10 (3H, d, *J*=6.0 Hz, H-6"')], and 9 additional protons (δ 3.98-3.24, 9H, H-2"'H-5"') from sugars. Additionally, the molecular weight of this compound was 624 based on the pseudomolecular ion peak at *m/z* 647.2 $[M+Na]^+$ in the ESI-MS (positive) spectrum. Compound **14** was suggested to be isorhamnetin rutinoside. Therefore, the ^{1}H-NMR data were compared with those of isorhamnetin rutinoside isolated from cactus pear (*Opunta ficus-indicava*), as reported previously (27) (Fig. 2). Finally, compound **14** was identified as isorhamnetin 3-*O*-rutinoside.

The 14 compounds were isolated from the 60% EtOH extracts of immature pear fruit. These compounds were identified as 5-*O*-*trans*-caffeoyl quinic acid methyl ester (**1**), malaxinic acid (**2**), 5-*O*-*trans-p*-coumaroyl quinic acid methyl ester (**3**), 5-*O*-*cis-p*-coumaroyl quinic acid methyl ester (**4**), 5-*O*-*trans-p*-coumaroyl quinic acid (**5**), *trans-p*-coumaric acid (**6**), methyl *cis-p*-coumarate (**7**), methyl *trans-p*-coumarate (**8**), 3,5-*O*-dicaffeoyl quinic acid (**9**), (-)-epicatechin (**10**), (*S*)-(+)-2-*cis*-abscisic acid (**11**), isorhamnetin 3-*O*-β-D-galactopyranoside (**12**), isorhamnetin 3-*O*-β-D-glucopyranoside (**13**), and isorhamnetin 3-*O*-α-L-rhamnopyranosyl (1→6)-*O*-β-D-glucopyranoside (**14**). Six compounds (**1**, **2**, **6**, **9**, **10**, and **13**) have already been identified from pear fruit peels (15). However, the other 8 compounds (**3**-**5**, **7**, **8**, **11**, **12**, and **14**) were identified for the first time from pear fruit. These 14 isolated compounds showed antioxidative activity in a guided DPPH radical scavenging assay during the purification procedure. However, most of the identified compounds were structurally elucidated as a mixture and/or of lower purity with trace amounts. Therefore, an antioxidative activity evaluation of the individual compounds will be conducted later.

In our previous studies (14,15), the presence of various phenolic compounds and coumaric acid derivatives including 7 phenylpropanoid malate derivatives from mature pear fruit peels of pear (*P. pyrifolia*) were reported. In this study, another 6 coumaric acid derivatives (**3**-**8**) were additionally isolated from immature pear fruit. Abscisic acid (**11**) is well known as a plant hormone and a drought stress response compound (28). Abscisic acid found in peanut exerts an inhibitory effect against seed germination (29). However, investigations on the function and role of abscisic acid contained in pears have not been performd. Therefore, a quantitative behavioral analysis of abscisic acid according to the cultivation method and growth stage of pears may provide important information for improving

pear quality. Compound **12-14** possessed a common isorhamnetin as an aglycone in their partial structures. Isorhamnetin acts as an anticancer, anti-inflammatory, and antioxidative activity substance (30). Therefore, the isorhamnetin glycosides (**12-14**) may contribute to various biological activities.

Low quality immature fruits are generally handpicked in the early stage after florescence to harvest high quality pears. Generally only 1 fruit of a cluster of 7-8 fruits remains after this process, and the handpicked immature fruits are discarded. We proposed the possibility of applying the discarded immature pear fruit in a previous report (18) based on the recovery of β-arbutin, which is one of the most abundant compounds in pear (*P. pyrifolia* Nakai) fruit peels and has been widely used as a whitening agent in cosmetic products. Interestingly, its content was highest in immature fruit, which was about 25-fold higher than the content in mature fruit (5,18). Although it is only one example, it might provide an application for the discarded immature pear fruit. In addition, the content of water in immature fruits (78.8%) was lower than that in mature fruit (87.3%).

We have clarified that Asian pears contain many biologically active compounds. These investigations at the molecular level offer very useful information on functionality and quality as well as plant physiology of pear fruit. Moreover, the results play a very important role in establishing the chemical profiles of pear constituents.

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References

1. Hahashi AA, Dashti S, Abdollahi H, Kermani MJ. Comparing vaccum *Agroinoculation* in two pear (*Pyrus communis* L.) cultivars 'Bartlett' and 'Harrow Delight'. Ann. Biol. Res. 3: 3200-3207 (2012)
2. Li X, Zhang JY, Gao WY, Wang Y, Wang HY, Cao JG, Huang LQ. Chemical composition and anti-inflammatory and antioxidant activities of eight pear cultivars. J. Agr. Food Chem. 60: 8737-8744 (2012)
3. Challice JS, Williams AH. Phenolic compounds of the genus *Pyrus*-II: A chemotaxonomic survey. Phytochemistry 7: 1781-1785 (1968)
4. Shakir I, Durrani Y, Hussain I, Qazi IM, Zeb A. Physicochemical analysis of apple and pear mixed fruit jam prepared from varieties grown in Azad Jammu and Kashmir. Pak. J. Nutr. 7: 177-180 (2008)
5. Cui T, Nakamura K, Ma L, Li JZ, Kayahara H. Analyses of arbutin and chlorogenic acid, the major phenolic constituents in oriental pear. J. Agr. Food Chem. 53: 3882-3887 (2005)
6. Chen J, Wang Z, Wu J, Wang Q, Hu X. Chemical compositional characterization of eight cultivars grown China. Food Chem. 140: 268-275 (2007)
7. Salta J, Martins A, Santos RS, Neng NR, Nogueira JMF, Justino J, Rajter AP. Phenolics composition and antioxidant activity of 'Rocha' pear and other pear cultivars-A comparative study. J. Fun. Food 2: 153-157 (2010)
8. Schieber A, Keller P, Carle R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. J. Chromatogr. 210: 265-273 (2001)
9. Tanrıöven D, Ekşi A. Phenolic compounds in pear juice from different cultivars. Food Chem. 93: 89-93 (2005)
10. Ferreira D, Guyot S, Marnet N, Delgadillo I, Renard CM, Coimbra MA. Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. *S. bartolomeu*) and changes after sun-drying. J. Agr. Food Chem. 50: 4537-4544 (2002)
11. Challice JS, Westwood MN. Phenolic compounds of the genus *Pyrus*. Phytochemistry 11: 37-44 (1972)
12. Lin LZ, Harnly JM. Phenolic compounds and chromatographic profiles of pear skins (*Pyrus* spp.). J. Agr. Food Chem. 5: 9094-9101 (2008)
13. Huang LJ, Gao WY, Li X, Zhao WS, Huang LQ, Liu CX. Evaluation of the *in vivo* anti-inflammatory effects of extracts from *Pyrus bretschneideri* Rehd. J. Agr. Food Chem. 58: 8983-8987 (2010)
14. Lee KH, Cho JY, Lee HJ, Ma YK, Kwon J, Park SH, Lee SH, Cho JA, Kim WS, Park KH, Moon JH. Hydroxycinnamoylmalic acids and their methyl esters from pear (*Pyrus pyrifolia* Nakai) fruit peel. J. Agr. Food Chem. 59: 10124-10128 (2011)
15. Lee KH, Cho JY, Lee HJ, Park KY, Ma YK, Lee SH, Cho JA, Kim WS, Park KH, Moon JH. Isolation and identification of phenolic compounds from pear (*Pyrus pyrifolia* Nakai) fruit peel. Food Sci. Biotechnol. 20: 1539-1545 (2011)
16. Oh HJ, Jeon SB, Kang HY, Yang YJ, Kim SC, Lim SB. Chemical composition and antioxidative activity of kiwifruit in different cultivars and maturity. J. Med. Food 40: 343-349 (2011)
17. Kumar S, Yadav P, Jain V, Malhota SP. Oxidative stress and antioxidative system in ripening ber (*Ziziphus mauritiana* Lam.) fruits. Food Technol. Biotechnol. 45: 453-459 (2011)
18. Cho JY, Park KY, Lee KH, Lee HJ, Lee SH, Cho JA, Kim WS, Shin SC, Park KH, Moon JH. Recovery of arbutin in high purity from fruit peels of pear (*Pyrus pyrifolia* Nakai). Food Sci. Biotechnol. 20: 801-807 (2011)
19. Takao T, Kitatani F, Sakata K. A simple method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Bioch. 58: 1780-1783 (1994)
20. Sadhu SK, Okuyama E, Fujimoto H, Ishiubashi M, Yesilada E. Postaglandin inhibitory and antioxidant components of *Cistus laurifolius*, a Turkish medicinal plant. J. Ethnopharmacol. 108: 371-378 (2006)
21. Lu YR, Sun Y, Yeap Foo L, McNabb WC, Molan AL. Phenolic glycosides of forage legume *Onobrychis viciifolia*. Phytochemistry 55: 67-75 (2000)
22. Chen YH, Chang FR, Lu MC, Hsieh PW, Wu MJ, Du YC, Wu YC. New benzyol glucosides and cytotoxic pterosin sesquiterpenes from *Pteris ensiformis* Burm. Molecules 13: 255-266 (2008)
23. Chung JH, Shin HC, Cho JY, Kang SK, Lee HJ, Shin SC, Park KH, Moon JH. Isolation and structural determination of free radical scavenging compounds from Korea fermented red pepper paste (*gochujang*). Food Sci. Biotechnol. 18: 463-470 (2009)
24. Duque Estrada GO, DE Souza ALF, Mendes da Silva JF, Antunes OAC. Revisiting *p*-coumarate esters production by heck reaction. Catal. Commun. 9: 1734-1738 (2008)
25. Milborrow BV. The conformation of abscisic acid by NMR and a revision of the proposed mechanism for cyclization during its biosynthesis. Biochem. J. 220: 325-332 (1984)
26. Guvenap Z, Demirezer LO. Flavonol glycosides from *Asperula arvensis* L. Turk. J. Chem. 29: 163-169 (2005)
27. Lee EH, Kim HJ, Song YS, Jin C, Lee KT, Cho J, Lee YS. Constituents of the stems and fruits of *Opuntia ficus-indica* var. *saboten*. Arch. Pharm. Res. 26: 1018-1023 (2003)
28. Reddy AR, Chaitanya KV, Vivekanandan M. Drought-induced

responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161: 1189-1202 (2004)
29. Hu B, Wan XR, Liu XH, Guo DL, Li L. Abscisic acid (ABA)-mediated inhibition of seed germination involves a positive feedback regulation of ABA biosynthesis in *Arachis hypogaea* L. Afr. J. Biotechnol. 9: 1578-1586 (2010)
30. Shinozaki K, Yamaguchi-Shinozaki K. Gene expression and signal transduction in water-stress response. Plant Physiol. 115: 327-334 (1997)